Conventional and genetically modified near-isoline *Zea mays* yield responses to pre-emergence isoxaflutole herbicide application

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Introduction and Background

The chemical herbicide active ingredient isoxaflutole was approved by the United States Environmental Protection Agency for use in the midwestern regions of the United States, including Ohio, in 1998. Isoxaflutole is a soil-applied, pre-emergence herbicide labeled for *Zea mays*, (L.) or corn, that controls weeds such as *Echinochloa crus-galli* (barnyardgrass), *Panicum dichotomiflorum* (fall panicum), *Setaria faberi* (giant foxtail), *Sicyos angulata* (burcucumber), *Solanum ptycanthum* (eastern black nightshade), *Ambrosia artemisiifolia* (common ragweed), *Datura stramonium* (jimsonweed), *Chenopodium album* (common lambsquarters, including atrazine-resistant biotypes), *Amaranthus retroflexus* (redroot pigweed), *Polygonum pennsylvanicum* (Pennsylvania smartweed), and *Abultilon theophrasti* (velvetleaf). Isoxaflutole kills and suppresses weeds by a unique mode of action, the inhibition of the 4-hydroxyphenylpyruvate dioxygenase enzyme, or 4-HPPD. This enzyme inhibition causes the reduction of plastoquinone, which acts as a cofactor of phytoene desaurase, a key enzyme in cartenoid biosynthesis, which in turn is vital in the production and maintenance of chlorophyll, carotenes, and other related photosynthetically important radiation-activated biochemicals (Lee et al. 1997). Isoxaflutole is also suspected to inhibit an enzyme or enzymes important in the metabolism of the aromatic amino acid, tyrosine. The apparent effects in plants are the bleaching of selected plant foliage, thus rendering the plant unable to utilize correct solar wavelengths for the photosynthetic process (Sprague et al. 1999), and plant stunting due to the subsequent irregular metabolism. The plant uses remaining nutrient reserves and is either stunted or killed essentially by starvation.

Before the discovery of isoxaflutole, the only other herbicide with a similar mode of action was clomazone, a pigment synthesis inhibiting herbicide for pre-emergence application weed control in soybeans. Isoxaflutole provides a new alternative (method) for broad-spectrum weed control in corn.

Agronomically, corn growth is best during times of low weed pressure from emergence to five weeks. After five weeks, weed pressure has a negligible effect on corn total yield (Anonymous 1996). Thus, control of weeds from corn emergence to five weeks is the goal for optimal corn production systems. Isoxaflutole exhibits extended environmental persistence in the ground, that is, isoxaflutole is chemically active for some time after application, and does not deteriorate or break down quickly on reaching the soil surface, actually for a period of about six weeks, as opposed to some pre-emergence herbicides, which become biochemically inactive as soon as two weeks after application in the soil solution. An additional property of isoxaflutole is that rainfall or irrigation "re-activates" the chemical salt, redistributing it back into the near-surface soil solution, and then making it available to control or suppress the growth of selected weed species seedlings in the six-week window of time after application. Too much water can cause the herbicide to leach from the upper soil profile, causing possible damage to corn plants by excessive uptake, and in severe cases, cause groundwater contamination. Isoxaflutole controls labeled problem weeds efficiently, with about 90% control when applied at small amounts, as the normal field rate is 1.5 oz/acre on the soil types present in this study, another feature facilitating chemical handling.

With the advent of genetically modified corn varieties comes a great potential for new herbicide treatments to control problem weeds that would otherwise injure or in some cases destroy conventional field corn. Roundup Ready corn varieties, for example, are resistant to the post-emergence application of glyphosate, an effective, non-selective systemic herbicide, and the active ingredient in commercial herbicides such as Roundup and Touchdown. Corn producers can essentially apply glyphosate, a chemical that effectively controls all plants, to their fields and eliminate yield-robbing weed pressure in one pass, saving time and money while preserving environmental quality. Similar studies have been published concerning broadleaf weed control and crop tolerance for imidazolinone herbicide resistant and susceptible corn using a variety of herbicides, but isoxaflutole was not included in the study (Sprague et al. 1997).

The European and southwest Corn Borers, destructive corn insect pests, are targets of a different sort of genetic enhancement that can be expressed in today's corn hybrids. The natural soil-borne bacteria *Bacillus thuringiensis* (B.t.) is able to produce a protein that is poisonous to these insects, and corn can be engineered to produce the toxin, thus eliminating the need for a producer to apply commercial insecticides with possible detrimental environmental effects. Both glyphosate resistance and B.t. trait expression in a cropping system can serve as tools in the implementation and maintenance of an integrated pest management (IPM) system, in reduction of chemical usage.

Problem Identification and Justification

Unfortunately, isoxaflutole can injure corn plants with the same mode of action as it can damage weed species if the corn is not planted more than 3.8 centimeters (1.5 inches) deep with firm topsoil packing, as corn is only partially resistant to isoxaflutole (Anonymous 1998). Isoxaflutole effects on corn include stunted growth and yield loss if the duration of corn leaf discoloration is greater than one week. Additionally, isoxaflutole might possibly affect genetically modified Zea mays differently than conventional, non-Roundup Ready and non-B.t. hybrids. Few scholarly papers or journal articles are available on such interactions, though isoxaflutole + Roundup Ready and B.t. combination programs have been used in the Midwestern states.

Objective

The objective of this study is to evaluate three near-isoline corn hybrids, conventional, glyphosate resistant, and insect resistant by *Bacillus thuringiensis,* for pleiotropic effects via reduced fitness due to the pre-emergence application of a commercial isoxaflutole herbicide.

Materials and Methods

The field study location was situated on the Champaign County Fairgrounds in Urbana, Ohio, which is approximately 45 miles west of Columbus. The soil of the study area was Crosby silt loam, with 0-2% slopes, and somewhat poorly drained. Plot area preparation was such to acquire a conventionally tilled seedbed; tillage consisted of moldboard plowing in the previous autumn, followed by secondary tillage in the spring. Isoxaflutole action is not exaggerated or inhibited by soil technique based on plant residue cover remaining on the soil surface (Wrucke et al. 1997). No-tillage soybeans were planted on the plot area in the previous year.

Inorganic fertilizers were all broadcast-applied; 350 pounds of 9-23-30 were applied with 270 pounds of 46-0-0, yielding 80.5 pounds of P_2O_5 equivalent, 105.0 pounds K_2O equivalent, and 155.7 pounds of inorganic nitrogen. An unknown proportion of cow and horse manure mixed with wheat straw was spread over the soil at the fairground plot area as a means of animal waste disposal. An analysis of the manure mixture was not available, nor was the rate on the fields. Thus, labile organic nitrogen compounds may still have been in soil solution at time of planting, in addition to small amounts of nitrogen remaining from the previous soybean crop (Eckert 2001). Soil tests for phosphorus and potassium were well within the sufficient range with fertilizer amendments described previously, 213.0 pounds and 450.2 pounds of P_2O_5 and K_2O equivalents, respectively.

DeKalb Genetics DK580, DK580RR (glyphosate resistant), and DK580BtY (B.t.) hybrids were selected for this study. All hybrids were near-isolines with 90% germination test ratings. Two herbicide treatments per hybrid, replicated three times yield a total of eighteen plots, each plot measuring 20'x100' in a completely randomized design, with herbicide treatment as a split plot. The field is assumed to be uniform in all aspects. The plots were sown using a John Deere 7000 Conservation planter on May 16th. The planting population was 30,000 seeds per acre at a planting speed of 4.5 miles per hour. The corn planter held four planting units at 30" widths. Since plots were laid out 20' wide to accommodate a 20' chemical spraying boom, eight rows with 100' length comprised a plot planting. The weather conditions recorded were overcast skies, a temperature of 75 degrees F, relative humidity of 80%, and a wind speed of 15 to 25 miles per hour with variable direction. During planting, however, weather conditions suddenly worsened, and immediately following the planting of the final plot, 0.28" of rain was measured in 20 minutes.

Spraying of the isoxaflutole and control herbicides was delayed three days until May 19th; weather conditions were more favorable with a temperature of 78 degrees F and calm winds, and the soil was dry enough to safely drive a tractor over the plot without compacting the soil surrounding the seeds. A 20' wide custom-built spraying setup was implemented for applying the herbicides to the plots at 15 gallons of spray solution per acre using TeeJet 8004 flat fan nozzles using a pressure of 40PSI at a ground speed of 5.5MPH. Isoxaflutole-treated plots received 1.5 oz per acre of Balance 75WDG, which is 75% isoxaflutole active ingredient in a dry form. Non-isoxaflutole treated plots were sprayed with 2.4 quarts per acre of Bicep 6L, which contains 3.3 pounds of metolachlor and 2.7 pounds of atrazine per gallon of solution. Bicep 6L is pre-emergence herbicide labeled for corn that controls many of the same weeds as Balance to eliminate or suppress weed-crop competition that could affect yield in the non-isoxaflutole treated plots. Plots with Roundup-Ready corn were likewise sprayed with 32 fluid ounces per acre of Roundup Ultra to simulate normal chemical program treatment of such corn, in addition to the particular pre-emergence applications of Balance and Bicep.

Plot harvesting for yield determination took place on September 23rd, 2000, by picking every ear of corn in two random 20' row lengths. Samples were sealed in plastic bags and transported to Columbus, where husks were removed, one plot sample at a time. Sample mass data was obtained with a Chatillon commercial milk scale. Ears were then selected at random and shelled by hand to determine grain moisture content with a Dickey-John GAC2000 Grain Analysis Computer. Grain yield was calculated with the following equation, derived from, assuming standard moisture of 15.5% and bushel weight of 56 pounds.

$$\text{Yield} = \frac{(100 - \%\text{moisture}) * (\text{pounds of ears}) * (90.439)}{(\text{row width (inches)}) * \text{row length (feet)} * \text{number of rows}} \qquad \text{Equation 1}$$

Note: The third factor, 90.439, in Equation 1 is a conversion factor assigned to calculating grain yield for ear corn. Collected weight and grain moisture raw data are included in Appendix A.

Data Analysis

The raw data was processed and analyzed by computer, using the DOS-platform MSTATC program developed by the Michigan State University Department of Crops and Soils. Analysis of variance (ANOVA) at the 0.050 alpha level was performed to calculate significance in yield data by herbicide treatment and hybrid. Significance at the P = 0.05 level was found between hybrids; no significant yield differences existed between herbicide treatments.

Table 1. Effect of hybrids and herbicide treatments on corn grain yield.

	Herbicide Treatment (Herb) (yield in bushels/acre)		
Hybrid (Hyb)	isoxaflutole	metolachlor + atrazine	Mean
DeKalb DK580	176.60	184.44	180.52
DeKalb DK580RR	200.89	183.41	192.15
DeKalb DK580BtY	162.50	175.96	169.23
Mean	179.99	181.27	
$LSD_{0.05}$ (Hyb)			13.31
$LSD_{0.05}$ (Herb)	NS		
$LSD_{0.05}$ (Hyb*Herb) = NS			

Note: Output from the MSTATC program is included in Appendix A.

Discussion:

Statistically significant yield differences were not observed between herbicide treatments, that is, the application of isoxaflutole herbicide in this experiment did not cause a yield depression. However, significant yield differences were noted between hybrids, even though all hybrids were near-isolines. These yield differences, notably, DK580RR over the conventional DK580 and the insect resistant DK580BtY, might possibly be attributed to extra genetic "baggage," like gene promoters, which are necessary for proper expression of the genetically inserted trait (Harrison 2001). Though a significant yield difference existed between hybrids, no significant interaction was observed between hybrids and herbicide treatments (hyb * herb).

Weather conditions, specifically temperature and rainfall, for the plot were conducive to exceptional pre-emergence herbicide action, and weed escapes were minimal. Control was enhanced because of cool and wet spring weather. Precipitation is necessary for good chemical infiltration of the soil-applied herbicides and cool temperatures slow weed germination and growth (Stachler 2001). The plots were nearly 100% free of weeds, with the exception of the emergence of a few *Circium arvense* (Canada thistle) plants, which are not labeled weeds for either Balance or Bicep. Winter annuals began to emerge in mid-September at harvesting, but these weeds were considered to be negligible as a yield-affecting variable because grain had reached physiological maturity and young winter annuals are not strong enough competitors for resources in mid-autumn to drying corn.

Conclusions

1. There is no significant yield difference caused by the pre-emergence use of isoxaflutole as compared to metolachlor + atrazine when applied correctly on any of the three hybrids studied.
2. Statistically significant yield differences were noted between hybrids, though they are near-isolines.
3. No significant interaction between hybrids and herbicides was observed.

Experimental and Practical Issues, Confounding Variables, and Further Discussion

Field studies are at the mercy of the weather, and the 2000 crop season was a relatively good one, producing some great yields; all plots yielded above 150 bushels/acre, and some plots above 200 bushels/acre. These yield figures suggest the presence of additional organic soil nitrogen in addition to the applied inorganic nitrogen, as nitrogen is very often the most limiting nutrient factor in plant yield, especially in organic fertilizer situations with previously high levels of phosphorus and potassium (Eckert, 2001). Weather conditions are a major factor in crop performance, and of course, different weather patterns affect yield magnitude observed. To eliminate some of the effects that a particular weather pattern imposes, the study should be repeated again, perhaps for two more years, and in more than one location. Further replications will help either reinforce or disprove preliminary conclusions drawn from one location and one year of data.

In addition, small plots and small samples can exaggerate calculated yields or otherwise not reflect true yield gathered for a 1,000-acre field, for example. Other factors, like soil type and fertility variability play a major role in determining final field yields, and are somewhat eliminated for small-plot collections of data. Larger plots in less homogeneous fields would yield more practical data to a producer.

Economic advantages in using one weed control product over another could be explored with these findings, after more replications are performed and evaluated. 1.5 ounces per acre applied of Balance would cost a producer $13.36, and 2.4 quarts per acre of Bicep would cost a producer $11.32 for the same weed control effects, assuming optimum conditions and performance, and only the presence of the same set of targeted problem weeds. If perennial problem weeds, like *C. arvense*, populations are high, post-emergence herbicides may be necessary to control them, increasing input costs to the producer. If perennial weeds persist, a Roundup Ready program implementing Balance or Bicep at a reduced rate may be advantageous to the producer, reducing annual weed pressure until post-emergent herbicide application. Extension specialists suggest that a reduced rate of Balance with photosynthesis inhibitors and shoot meristem inhibitor herbicides, such as atrazine, acetochlor, and metolachlor, could prove to be effective annual weed control as well. Michigan State researchers evaluated weed and crop responses with isoxaflutole and tank-mixes, but not reduced-rate usage with atrazine, acetochlor, and metolachlor on transgenic corn hybrids (Sprague et al. 1999). Isoxaflutole is available in a pre-mix with flufenacet in a product from Bayer called "Epic." This product could be added in the evaluation of transgenic corn hybrid yield and herbicide interactions, as could stacked-gene corn hybrids than express both glyphosate resistance and B.t. insect resistance. Different types of proteins produced by *Bacillus thuringiensis* exist, including the infamous StarLink corn genetic makeup, and can be studied in further investigations.

Finally, the Aventis Corporation has introduced a new formulation of Balance for 2001, Balance Pro, a 4L liquid formulation of isoxaflutole with 4 pounds of active ingredient per gallon of solution, to facilitate applicator handling. This liquid formulation eliminates problems with water-dispersible granule formulations, as sometimes the product used does not dissolve in spray tank without 15 minutes of vigorous agitation, creating unequal concentrations of product in solution, possibly causing overdose and injury to corn and lack of weed control elsewhere. Another experiment should be run to determine if yield is affected by use of the WDG formulation or liquid formulation, should Aventis stop production of WDG formulation. Bicep, too, is available in different formulations and ratios of atrazine and metolachlor, and at the time of this paper's conclusion, the 6L formulation of Bicep is no longer available.

The 2001 DeKalb seed catalog lists hybrids and agronomic characteristics, as well as an herbicide tolerance chart for producers to reference (Anonymous, 2001). Further investigation and replications in this sort of research may prove to be useful to corn breeders and field agronomists when evaluating hybrids and making sound herbicide recommendations for producers.

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Appendix A: Sample moisture content and weight data collected September 23, 2000 and MSTATC Output

	Plot ID	Moisture	Weight	Yield (bu/ac)
DK580 w/Balance	2	26.7	27.8	153.58
	13	22.0	32.1	188.70
	16	20.0	31.1	187.51
Average		22.9	30.3	176.6
DK580 w/Bicep	4	23.5	36.2	208.71
	7	25.7	31.2	174.71
	11	24.1	29.7	169.89
Average		24.4	32.4	184.4
DK580RR w/Balance	1	22.8	35.7	207.71
	3	20.3	32.0	192.21
	15	21.8	34.4	202.74
Average		21.6	34.0	200.9
DK580RR w/Bicep	4	23.5	36.2	208.71
	10	20.7	29.0	173.22
	12	17.9	27.2	168.30
Average		20.7	30.8	183.4
DK580BtY w/Balance	8	25.6	27.5	154.20
	14	24.6	27.5	156.27
	18	19.0	29.0	177.03
Average		23.1	28.0	162.5
DK580BtY w/Bicep	5	23.5	32.5	187.38
	9	17.7	29.1	180.50
	17	22.8	27.5	160.00
Average		21.3	29.7	176.0

Output from MSTATC program

```
Data file:   KEITH
Title:       keith honors

Function: FACTOR

Experiment Model Number 2:
     Completely Randomized Design for Factor A, Factor B
     is a Split Plot
Data case no. 1 to 18.

Factorial ANOVA for the factors:
     Replication (Var 2: REP) with values from 1 to 3
     Factor A (Var 1: HYBRID) with values from 1 to 3
     Factor B (Var 3: TRT) with values from 1 to 2
```

Var. 1 – HYBRID: 1 = DK580, 2 = DK580RR, 3 = DK580BtY

Var. 3 – TRT: 1 = Balance 75WDG @ 1.5 oz/acre, 2 = Bicep 6L@ 2.4qt/acre

Variable 4: YIELD

Grand Mean = 180.632   Grand Sum = 3251.370   Total Count = 18

T A B L E   O F   M E A N S

2	1	3	4	Total
*	1	*	180.517	1083.100
*	2	*	192.148	1152.890
*	3	*	169.230	1015.380
*	*	1	179.994	1619.950
*	*	2	181.269	1631.420
*	1	1	176.597	529.790
*	1	2	184.437	553.310
*	2	1	200.887	602.660
*	2	2	183.410	550.230
*	3	1	162.500	487.500
*	3	2	175.960	527.880

A N A L Y S I S   O F   V A R I A N C E   T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	2	1575.870	787.935	5.9145	0.0381
-3	Error	6	799.332	133.222		
4	Factor B	1	7.309	7.309	0.0162	
6	AB	2	814.798	407.399	0.9008	
-7	Error	6	2713.524	452.254		
	Total	17	5910.832			

Coefficient of Variation: 11.77%

$s_{\bar{y}}$ for means group 2: 4.7121 Number of Observations: 6

$s_{\bar{y}}$ for means group 4: 7.0888 Number of Observations: 9

$s_{\bar{y}}$ for means group 6: 12.2781 Number of Observations: 3

Data File : KEITH
Title : keith honors

Case Range : 41 - 43
Variable 4 : YIELD
Function : RANGE

Error Mean Square = 133.2
Error Degrees of Freedom = 6
No. of observations to calculate a mean = 9

Least Significant Difference Test
LSD value = 13.31 at alpha = 0.050

Original Order				Ranked Order			
Mean	1 =	180.5	AB	Mean	2 =	192.1	A
Mean	2 =	192.1	A	Mean	1 =	180.5	AB
Mean	3 =	169.2	B	Mean	3 =	169.2	B